植物研究雑誌
J. Jpn. Bot.
72: 93–109 (1997)

Pharmacognostical Studies on the Sino-Japanese Crude Drugs "Huajiao (花椒)" and "Sansho (山椒)" (Part 4)
Determination of Botanical Origin of Chinese Crude Drug "Jiaomu (椒目)" by Scanning Electron Microscopy

Yu-Ping LIU[a], Chikashi ITO[b], Katsuko KOMATSU[b], Tadato TANI[c], Dai-Wen SHI[d] and Tsuneo NAMBA[b]

[a]Institute of Chinese Materia Medica, China Academy of Traditional Chinese Medicine, Beijing, 10700 CHINA;
[b]Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University, 2630, Sugitani, Toyama, 930-01 JAPAN;
[c]Research Labs. for High Quality of Life, Sumitomo Metal Industries, Ltd., 3-5, Hikari-Dai, Seika-Cho, Soraku-Gun, Kyoto, 619-02 JAPAN;
[d]School of Medicine, Shanghai Medical University, Shanghai, 200032 CHINA

(Received on August 2, 1996)

"Jiaomu," a traditional Chinese medicine used as a diuretic, is derived from the seeds of the genus *Zanthoxylum*, the family Rutaceae, and of the same origin as "Huajiao." On the other hand, it is difficult to identify *Zanthoxylum* seeds because of their similar external morphology and the difficulty of sectioning them to observe the inner structure. In this paper, in order to develop a method for the identification of "Jiaomu," we report a new anatomical method using a scanning electron microscope and an image analysis system on the seeds of 16 species, three varieties and one form, collected from China, Japan and Nepal. The results showed that 16 species and varieties could be distinguished from each other by the following characteristics: number of layers of epidermal cell, ratio of radial diameter to tangential diameter of epidermal cell, shape of closed curve which was drawn in between epidermis and sclerenchyma tissue (its degree of roughness shown by SFC value) ratio of average thickness of outer mesophyll to inner mesophyll, ratio of occupation of epidermis to outer seed coat, ratio of thickness of outer seed coat to the radius of transection of seed, etc. The commercial samples of Shanghai and Datong (Shanxi) market were the seed of *Z. bungeanum* and that of Huhehaote (Inner Mongolia) market was a mixture of seeds and pericarps of *Z. schinifolium*.

(Continued from Nat. Med. **51**: 1997)

Introduction

"Jiaomu" was first reported in "Ben-Cao-Jing-Ji-Zhu (502)" with the diuretic activity and it is distinguished from "Shujiao (蜀椒)". According to the "Manual of Chinese Medicines (1961)" and "Chinese Materia Medica (1977)" the botanical origin of "Jiaomu" has been regarded as the seeds of *Zanthoxylum bungeanum* and *Z. schinifolium*. At the same time, in some other literatures the botanical origin of "Jiaomu" has been mentioned as the seeds of *Z. simulans* (Quanguoyao Zhongcaoyao Huibian Editorial Office 1978, Jiangsu Institute of Botany et al. 1991, Manual of Medicinal Plants of Zhejiang Editorial Office 1980, Handbook of Medicinal Herbs of

Ningxia Editorial Office 1971, Handbook of Medicinal Herbs of Shandong Editorial Office 1970, Economic Plants of Shandong Editorial Office 1978, Institute of Chinese Medicine, Branch Office in Shanxi, Chinese Academy of Medical Science 1961), *Z. armatum* (Quanguoyao Zhongcaoyao Huibian Editorial Office 1978, Manual of Medicinal Plants of Zhejiang Editorial Office 1980, Institute of Chinese Medical and Pharmaceutical Sciences 1986), *Z. tibetanum* (Quanguoyao Zhongcaoyao Huibian Editorial Office 1978, Jiangsu Institute of Botany et al. 1991, Health Bureau of Autonomous Region of Xizang et al. 1971), and *Z. dissitum* (Quanguoyao Zhongcaoyao Huibian Editorial Office 1978), besides two origins mentioned above. Therefore, the botanical origin of the commercial sample is not clear (Table 1). In addition to this, it is very difficult to identify the botanical origin by the internal structure of the seeds of *Zanthoxylum* plants, since seeds are very hard and can't be cut by knife to prepare slices for the optical microscopic study. Regarding to these difficulties, it was aimed to study with a scanning electron microscope (Komatsu 1995). In the present paper, we wish to discuss the comparative study on the seed structures of 16 species, three varieties and one form collected from China, Japan and Nepal which we could distinguish in several parameters with a scanning electron microscope and an image analyzer.

Experimental

I. Materials

Plant specimens for comparison and crude drug samples are shown in Table 2.

All samples were preserved in the Museum of Materia Medica, Analytical Research Center for Ethnomedicines, Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University (TMPW) for the reference.

Table 1. Botanical Origin of "Jiaomu" mentioned in the references

	Reference No.*																	
Zanthoxylum Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Z. bungeanum	•	•	•	•	•	•	•	•	•	•	•							
Z. schinifolium	•	•	•			•						•						
Z. simulans			•	•								•	•	•	•	•		
Z. armatum			•									•					•	
Z. tibetanum			•	•														•
Z. dissitum			•															

*1: Manual of Chinese Medicines, 2: Chinese Materia Medica, 3: The Compilation of Chinese Medicinal Herbs, 4: Xinghua Bencao Gangyao, 5: The Authentic and Superior Medicinal Herbals in China, 6: Chinese Medicinal Herbs of Hebei, 7: Chinese Medicinal Herbs of Shanxi, 8: The Compilation of Medicinal Herbs Produced in Chengdu Area of Sichuan Prov., 9: Chinese Medicinal Herbs of Anhui, 10: Handbook for Diagnoses of Chinese Medicinal Herbs of Hubei, 11: Manual of Materia Medica of Fujian, 12: Manual of Medicinal Plants of Zhejiang, 13: Handbook of Chinese Medicinal Herbs of Ningxia, 14: Handbook of Chinese Medicinal Herbs of Shandong, 15: Economic Plants of Shandong, 16: Manual of Shanxi Medicines, 17: Manual of Medicinal Plants of Guangxi, 18: The Useful Materia Medica of Tibet.

II. Methods

1. Observation with a scanning electron microscope

a) Instruments: JSM-5300LV (JEOL). Measurement condition: accelerate voltage 15 KV or 20 KV.

b) Sample preparation: The sample was treated with 70% KOH for 3 min. and after neutralization with dilute acetic acid it was divided into halves by a knife. Thus prepared sample was spattered with gold of 200Å in thickness.

2. Detail analysis with an image analysis system

a) Instrument: High speed color image analysis system SP-500 SET (Olympus Optical Co., Ltd. and Nippon Avionics).

b) Measurement: The seeds were cut into two halves and the cut surface was observed by scanning electron microscope to take complete picture. The picture was traced out into the tracing paper and expanded. After calibration of the pixels to unit length, the image of picture was fed into the image processor through camera control unit under color photo lamp (Mitsubishi, FL10N-EDL). The image was binarized by the brightness level slicing methods. The binary image was corrected exactly and utilized for the analysis. The measurement parameters were an average of radius of seed (CRA), area (Aepo) and average thickness (THKepo) of epidermis, area (Asdo) and average thickness (THKsdo) of outer seed coat, average thickness of inner seed coat (THKsdi) and seed coat (THKsd) and the wavy pattern (SFC) formed in between epidermis and sclerenchyma tissue. The SFC value was calculated by the following equation:

SFC (roughness) = $\text{Perimeter}^2/4\pi \times \text{Area}$

III. External morphology of *Zanthoxylum* seeds

1. General morphology: Ellipsoidal to spherical, the outer surface was shining black and most of them were smooth, in some cases they were wrinkled.

2. The morphological characters of the seeds of each species for comparison are shown in Table 3.

IV. Anatomical characteristics of *Zanthoxylum* seeds (Corner 1976)

1. General morphology (Fig. 3)

The section of seed was oval or ovate (A_1). The seed coat was distinguished into outer and inner seed coat. Outer seed coat consisted of epidermis and sclerenchyma tissue. The epidermal cells were found as palisade tissue. In case of single epidermal layer the ratio of radial diameter and tangential diameter (R/T) of epidermal cell was measured at the valley of the sclerenchyma. If epidermal cell layer were two or more, R/T was measured in cells of the outer layer at the valley. Number of epidermal cell layers and arrangement were different from species to species. Sclerenchyma of outer seed coat was divided into outer mesophyll [layer of cells of about same diameter, outer layer] and inner mesophyll [layer of tangentially elongated cells, inner layer]. Each species was different in the ratio of average thickness of outer and inner mesophyll (THKsco/sci). The boundary between epidermis and sclerenchyma tissue was wavy and its degree of roughness (SFC), the ratio of area of epidermis (Aepo/sdo) and thickness of epidermis (THKepo/sdo) in outer seed coat, the ratio of average thickness of outer seed coat to average radius of seed (THKsdo/CRA) varied in different species. Inner seed coat was made by large and somewhat tangentially elongated cells with 3–6 cell layers and but in case, 1–2 cell layers of small cells were followed (shown in the parenthesis). The ratio of thickness of inner seed coat to whole seed coat (THKsdi/sd) was found to be varied from species to species.

Table 2. Collection data of the materials on use

i) Chinese crude drugs "Jiaomu" obtained from markets of China and Japan

Name		Market	Date of Purchase	TMPW No.*
Jiaomu	China:	Shanghai Chinese drug Co., Shanghai	Jul., 1994	13926
		Pingcheng drug store, Datong, Shanxi	Jul., 1994	15337
	Japan:	Tochimoto Tenkaido Co., Ltd., Osaka	Mar., 1994	15459
Chuan-jiaomu	China:	Mongolia Drug Store, Huhehaote, Inner Mongolia Autonomous Region	Jul., 1994	15113
Buguzhi	China:	Hutai management of Qinghaiseng Chinese Drug Co., Xining, Qinghai	Jul., 1993	13658

ii) Plant Materials

Locality	Collector	Date	Specimen No.
Zanthoxylum bungeanum Maxim.			
Herbal Garden, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Japan	C. Ito	Oct., 1991	183
Cheng Co., Gansu Prov., China	Y. P. Liu	Aug., 1992	92041
Z. simulans Hance			
Herbal Garden, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Japan	C. Ito	Oct., 1991	197
Z. podocarpum Hemsl.			
Dongyushan, Shanghai, China	Q. B. Xiong	Sep., 1989	8906017
Z. piasezkii Maxim.			
Wenchuan Co., Sichuan Prov., China	Y. P. Liu	Sep., 1992	359
Z. armatum DC. var. *armatum*	T. Namba et al.	Aug., 1983	832
Z. armatum DC. var. *subtrifoliatum* (Franchet) Kitamura			
Fengjie Co., Sichuan Prov., China	Y. P. Liu	Sep., 1992	369
Puge Co., Sichuan Prov., China	G. C. Zhou et al.	Jul., 1992	92092

Z. acanthopodium var. *timbor* Hook. f. Huili Co., Sichuan, China	G. C. Zhou et al.	Aug., 1992	92022
Z. dimorphophyllum Hemsl. var. *dimorphophyllum* Qingcheng shan, Doujiangyan, Sichuan Prov., China	Y. P. Liu	Sep., 1992	362, 363
Z. dimorphophyllum Hemsl. var. *spinifolium* Rehd. & Wils. Nanchong Co., Sichuan Prov., China	G. Y. Zhong	Aug., 1977	27
Z. piperitum DC. f. *piperitum* Herbal Garden, Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Japan	C. Ito	Oct., 1991	178
Z. piperitum DC. f. *inerme* Makino (Asakura-zansho) Wakayama Pref., Japan	C. Ito	Oct., 1991	219
Z. piperitum DC. f. *inerme* Makino (Budo-zansho) Wakayama Pref., Japan	C. Ito	Sep., 1991	218
Z. schinifolium Sieb. & Zucc. Dandong, Liaoning Prov., China	Q. B. Xiong	Oct., 1987	871025
Z. ailanthoides Sieb. & Zucc. Dandong, Liaoning Prov., China	Q. B. Xiong	Nov., 1989	891109
Z. molle Rehd. Tianmushan, Zhejian Prov., China	Q. B. Xiong	Nov., 1988	881119
Z. avicennae (Lam) DC. Nannin, Guangxi Zhuangzu Autonomous Region, China	Q. B. Xiong	Oct., 1988	881026
Z. esquirolii Lévl. Lushan Co., Sichuan Prov., China	G. C. Zhou	Sep., 1978	780589
Z. stenophyllum Hemsl. Nanchong Co., Sichuan Prov., China	G. Y. Zhong	Aug., 1977	33
Z. nitidium (Roxb.) DC. Nannin, Guangxi Zhuangzu Autonomous Region, China	Q. B. Xiong	Nov., 1989	891105

Z. dissitum Hemsl.			
Nandan, Guangxi Zhuangzu Autonomous Region, China	Q. B. Xiong	Oct., 1988	8808015

*Crude drugs and plant materials are preserved in the Museum of Materia Medica, Analytical Research Center for Ethnomedicines, Research Institute for Wakan-Yaku, Toyama Medical and Pharmaceutical University (TMPW).

2. Anatomical characteristics of each species

1. Zanthoxylum bungeanum Maxim. (Figs. 1-A, 3-A)

Epidermis was made by 1–2 cell layers (Fig. 3-A_2), R/T was 3.0–5.0, the boundary between epidermis and sclerenchyma tissue was wavy and SFC value was observed as 1.23±0.05. THKsco/sci was 2.1–3.0 in sclerenchyma, the epidermal cells occupied 43.4 ± 6.3% of outer seed coat in area, the percentage of thickness occupying (THKepo/sdo) was 39.7 ± 6.3%. THKsdo/CRA was 26.9 ± 2.4%. Inner seed coat was made by 2–3 (1) cell layers and occupied 11.4 ± 1.1% of the seed coat in thickness.

2. Zanthoxylum simulans Hance (Figs. 1-B, 3-B_2)

Epidermis was made by 2 cell layers with slightly shorter in radial than *Z. bungeanum*, and R/T was 2.0–3.2. The boundary between epidermis and sclerenchyma tissue was wavy and SFC value was observed as 1.22 ± 0.01. THKsco/sci was 3.0–3.8 in sclerenchyma. Aepo/sdo, THKepo/sdo and THKsdo/CRA were almost similar with that of *Z. bungeanum*. Inner seed coat was made by 3–4 (1) cell layers.

3. Zanthoxylum armatum DC. var. armatum (Fig. 1-C)

Zanthoxylum armatum was closely related to *Z. bungeanum*. The boundary between epidermis and sclerenchyma tissue was wavy with slightly smooth and SFC value was observed as 1.18. THKsdo/CRA was 32.1%. Inner seed coat was made by 3–4 (1) cell layers.

4. Zanthoxylum armatum var. subtrifoliatum (Fr.) Kitamura (Fig. 3-C_2)

It was found to be similar with that of *Z. armatum* var. *armatum*. Epidermal cells were slightly long with the R/T as 3.6–5.4. Inner seed coat occupied 16.9 ± 3.4% of seed coat and it was higher than that of *Z. armatum* in thickness.

5. Zanthoxylum acanthopodium DC. var. timbor Hook. f. (Figs. 1-D, 3-D_2)

Epidermis was made by single cell layer. Aepo/sdo and THKepo/sdo were 34.0 ± 5.0 and 30.3 ± 4.8%, respectively, which were lower than other species. Inner seed coat was made by 3–5 (1) cell layers and occupied 16.5 ± 2.3% of seed coat.

6. Zanthoxylum podocarpum Hemsl. (Figs. 1-E, 3-$E_{2,3}$)

Epidermis was made by 1–2 cell layers. The boundary between epidermis and sclerenchyma tissue was slightly wavy or nearly straight, and SFC value was observed as 1.15 ± 0.01. Inner seed coat was made by more cell layers than other species, which was found to be 5–7 (1) cell layers (Fig. 3-E_3).

7. Zanthoxylum dimorphophyllum Hemsl. var. dimorphophyllum (Fig. 1-F, 3-$F_{2,3}$)

Epidermis was made by single cell layer and R/T was 1.6–2.5. The boundary between epidermis and sclerenchyma tissue was almost straight. The SFC value was observed as 1.15 ± 0.02. Inner mesophyll of sclerenchyma was absent. Seed coat was thin and THKsdo/CRA was 9.3 ± 0.7%. Inner seed coat was

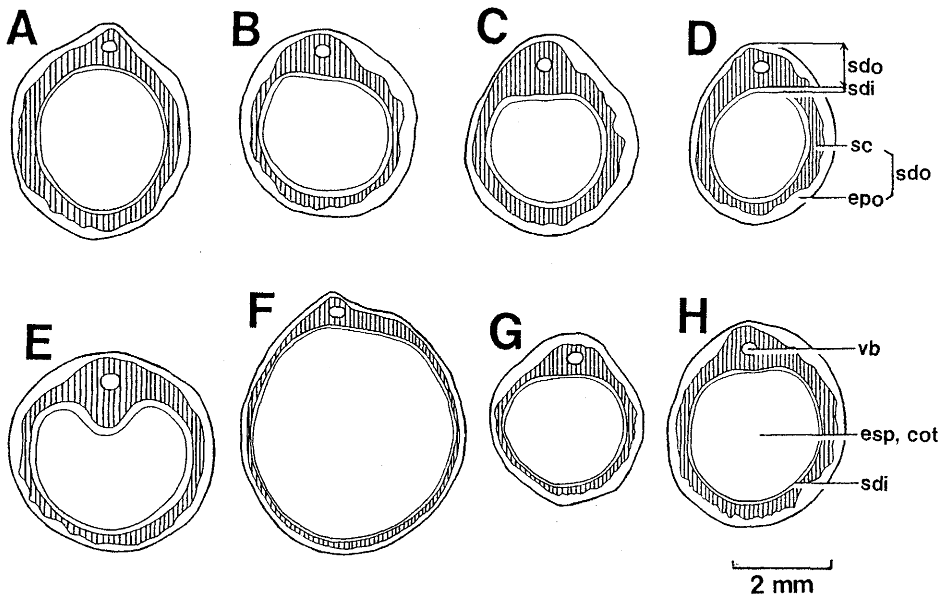

Fig. 1. Transection of seeds of subgen. Zanthoxylum species. A, *Z. bungeanum*; B, *Z. simulans*; C, *Z. armatum*; D, *Z. acanthopodium* var. *timbor*; E, *Z. podocarpum*; F, *Z. dimorphophyllum*; G, *Z. piasezkii*; H, *Z. piperitum*. (**cot**, cotyledon; **epo**, outer epidermis; **esp**, endosperm; **sc**; sclerenchyma; **sdi**, inner seed coat; **sdo**, outer seed coat; **vb**, vascular bundle).

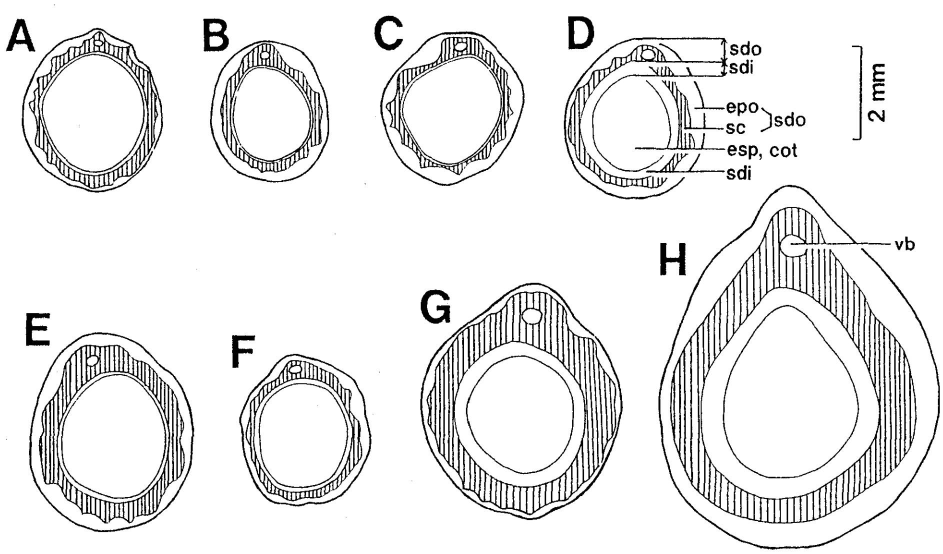

Fig. 2. Transection of seeds of subgen. Fagara species. A, *Z. schinifolium*; B, *Z. ailanthoides*; C, *Z. avicennae*; D, *Z. molle*; E, *Z. stenophyllum*; F, *Z. esquirolii*; G, *Z. nitidum*; H, *Z. dissitum*. (**cot**, cotyledon; **epo**, outer epidermis; **esp**, endosperm; **sc**; sclerenchyma; **sdi**, inner seed coat; **sdo**, outer seed coat; *vb*, vascular bundle).

made by 2–3 (1) cell layers and ratio of occupation is high and THKsdi/sd was 15.9 ± 3.0%.

8. Zanthoxylum dimorphophyllum var. spinifolium Rehd. & Wils.

It was almost similar with that of *Z. dimorphophyllum* var. *dimorphophyllum* but epidermal cells were relatively short, with the R/T as 1.0–1.6. Inner seed coat was made by 3–4(1–2) cell layers.

9. Zanthoxylum piasezkii Maxim. (Figs. 1-G, 3-$G_{2,3}$)

Epidermis was made by single cell layer with the R/T 2.8–6.7. The boundary between epidermis and sclerenchyma tissue was slightly wavy and the SFC value was observed as 1.18 ± 0.04. Seed coat was thin and THKsdo/CRA was 23.8 ± 0.8%. Inner seed coat was made by 2–3 (1) cell layers.

10. Zanthoxylum piperitum (L.) DC. f. piperitum (Fig. 1-H)

It was similar to that of *Z. bungeanum*. Epidermis was arranged nearly alternately both 1 and 2 cell layers, and R/T was 4.0–6.5. Inner seed coat was made by 4–5 (1) cell layers and the ratio of occupation was low and THKsdi/sd was 8.6%.

11. Zanthoxylum piperitum f. inerme Makino (Asakura-zansho and Budo-zansho)

Epidermis was shorter than that of *Z. piperitum* f. *piperitum* with the R/T as 2.0–4.0. Inner seed coat was made by 3–4 (1) cell layers. Asakura-zansho and Budo-zansho were distinguished by the ratio of thickness of outer mesophyll to inner mesophyll in sclerenchyma (THKsco/sci), the former indicating 4.3–5.5 and the later about 2.0.

12. Zanthoxylum schinifolium Sieb. & Zucc. (Figs. 2-A, 4-A)

Seeds were small. Epidermis was made by 1–2 cell layers, R/T was 3.5–4.5, the boundary between epidermis and sclerenchyma tissue was deeply wavy and SFC value was observed as 1.36 ± 0.03. The seed coat was thin and THKsdo/CRA was 18.7 ± 3.5%. Inner seed coat was made by 5 (1) cell layers and ratio of occupation in seed coat was slightly high and THKsdi/sd was 13.4 ± 0.7%.

13. Zanthoxylum ailanthoides Sieb. & Zucc. (Figs. 2-B, 4-B)

Seeds were small. Epidermis was made by 2 cell layers in thick part and single layer in thin part. Epidermis was the longest of all, R/T was 5.0–11.0, the boundary between epidermis and sclerenchyma tissue was deeply wavy and SFC value was observed as 1.27 ± 0.01. A large portion of the outer seed coat was occupied by the epidermis. Aepo/sdo and THKepo/sdo were 52.2 ± 0.1 and 47.9 ± 0.1%. Inner seed coat was made by 2–3 (1) cell layers. The ratio of occupation was low with the THKsdi/sd as 8.3 ± 0.3%.

14. Zanthoxylum avicennae (Lam.) DC. (Figs. 2-C, 4-C)

Epidermis occupied a large portion of the outer seed coat as found in *Z. ailanthoides*. But, R/T was 2.5–3.5, which was different from *Z. ailanthoides*. Epidermis was made by 2 cell layers. The wave of the boundary between epidermis and sclerenchyma tissue was the deepest of all and SFC value was observed as 1.38 ± 0.09. Inner seed coat was made by 5–6 (1) cell layers and the ratio of occupation was low with the THKsdi/sd as 10.6 ± 1.7%.

15. Zanthoxylum molle Rehd. (Figs. 2-D, 4-D)

Epidermis was made by single layer, and slightly long in radial as in *Z. ailanthoides*. The boundary between epidermis and sclerenchyma tissue was deeply wavy. A large portion of the seed coat was occupied by the epidermis as found in *Z. ailanthoides*. Number of the cell layers in the inner seed coat was large which was found to be 6–7 (1) cell layers. The ratio of occupation of inner seed coat in seed coat was high with the THKsdi/sd as 29.8 ± 5.3%. THKsdo/CRA was slightly low, 26.5 ± 1.4%.

Table 3. External morphology on seeds of *Zanthoxylum* species

	Subgenus	Zanthoxylum									
	Species	*Z. bungeanum*	*Z. simulans*	*Z. armatum* var. *armatum*	*Z. armatum* var. *subtrifoliatum*	*Z. acanthopodium* var. *timbor*	*Z. podocarpum*	*Z. dimorphophyllum* var. *dimorphophyllum*	*Z. dimorphophyllum* var. *spinifolium*	*Z. piasezkii*	*Z. piperitum*
Elements	Japanese name	Fuyu-Zansho									Sansho
	Locality	China, Japan	Japan	Nepal	China	China	China	China	China	China	Japan
Shape		ellipsoidal	globose-ellipsoidal	ellipsoidal	nearly globose	globose-ellipsoidal	ellipsoidal	nearly globose	nearly globose	nearly globose	globose
Length (mm)		3.5–4.7	3.2–3.8	3.4–4.0	3.0–3.5	2.7–3.7	ca. 4.4	3.9–4.8	3.6–4.3	2.5–2.9	4.0–4.7
Width (mm)		2.5–3.2	2.7–3.1	2.5–2.9	2.8–3.3	2.1–2.8	3.0–3.3	3.8–4.3	3.4–3.8	2.3–2.6	3.0–3.6
Surface	luster	+	+	+	+	+	+	+	+	+	+
	wrinkle	–	–	–	–	+	–	–	–	–	–
Number of seed/fruit		1(2)	1(2)	1(2)	1 or 2	1	1	1	1	1	1

	Subgenus	Zanthoxylum		Fagara							
	Species	*Z. piperitum* f. *inerme*		*Z. schinifolium*	*Z. ailanthoides*	*Z. avicennae*	*Z. molle*	*Z. stenophyllum*	*Z. esquirolii*	*Z. nitidum*	*Z. dissitum*
Elements	Japanese name	Asakura-zansho	Budo-zansho	Inu-zansho	Karasu-zansho						
	Locality	Japan	Japan	China	China	China	China	China	China	China	China
Shape		nearly globose	globose-ellipsoidal	nearly globose	globose-ellipsoidal	reniform	nearly globose	ellipsoidal	nearly globose	globose	nearly globose
Length (mm)		4.1–4.7	4.3–5.0	3.1–4.0	2.7–2.8	2.9–3.4	2.0–3.6	4.0–4.4	3.2–3.9	4.3–4.9	6.3–6.7
Width (mm)		3.1–3.8	3.1–3.6	2.6–3.0	2.2–2.5	2.4–2.6	1.6–2.7	ca. 3.3	2.7–3.5	3.8–4.2	5.0–5.9
Surface	luster	+	+	+	+	+	+	+	+	+	+
	wrinkle	–	–	+	–	+	+	–	–	–	–
Number of seed/fruit		1	1	1	1	1	1	1	1	1	1

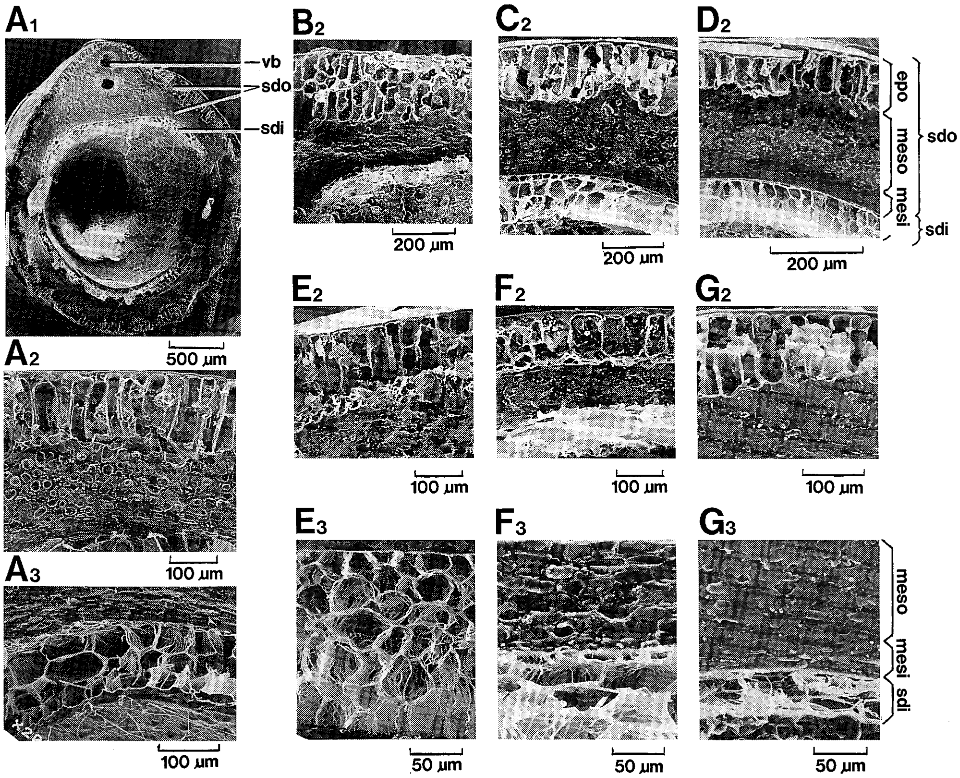

Fig. 3. Secondary Electron Images on the Transection of Seeds of Subgen. Zanthoxylum Species. A, *Z. bungeanum*; B, *Z. simulans*; C, *Z. armatum* var. *subtrifoliatum*; D, *Z. acanthopodium* var. *timbor*; E, *Z. podocarpum*; F, *Z. dimorphophyllum*; G, *Z. piasezkii* (1, whole seed; 2, epidermis and outer mesophyll; 3, inner mesophyll and inner seed coat) (**epo**, outer epidermis; **mesi**, inner mesophyll; **meso**, outer mesophyll; **sdi**, inner seed coat; **sdo**, outer seed coat; **vb**, vascular bundle).

16. Zanthoxylum stenophyllum Hemsl. (Figs. 2-E, 4-E)

Epidermis was made by 2 layers with radially short cells of the R/T 1.4–1.6. The boundary between epidermis and sclerenchyma tissue was similar to that of *Z. schinifolium* with the deep wave, but epidermis occupied a large portion in outer seed coat. Aepo/sdo, THKepo/sdo and THKsdo/CRA were 44.5, 39.8 and 32.4%, respectively. Inner seed coat was made by 5–6 (1) cell layers and THKsdi/sd was 14.8%.

17. Zanthoxylum esquirolii Lévl. (Figs. 2-F, 4-F)

It was more or less similar with that of *Z. stenophyllum*. The epidermal cells of the first layer of the two layers were about square in shape, while those of the inner layer were radially long. Ratio of occupation of epidermis in outer seed coat was higher than *Z. stenophyllum*. Aepo/sdo and THKepo/sdo were 51.3 and 48.2%, respectively. THKsdo/CRA was 21.8% which was lower than *Z. stenophyllum*. Inner seed coat was made by 3–5 (1–2) cell layers and THKsci/sd was 19.9%.

18. Zanthoxylum nitidum (Roxb.) DC. (Figs. 2-G, 4-G)

Epidermis was made by single layer. The boundary between epidermis and sclerenchyma tissue was deeply wavy and SFC value was

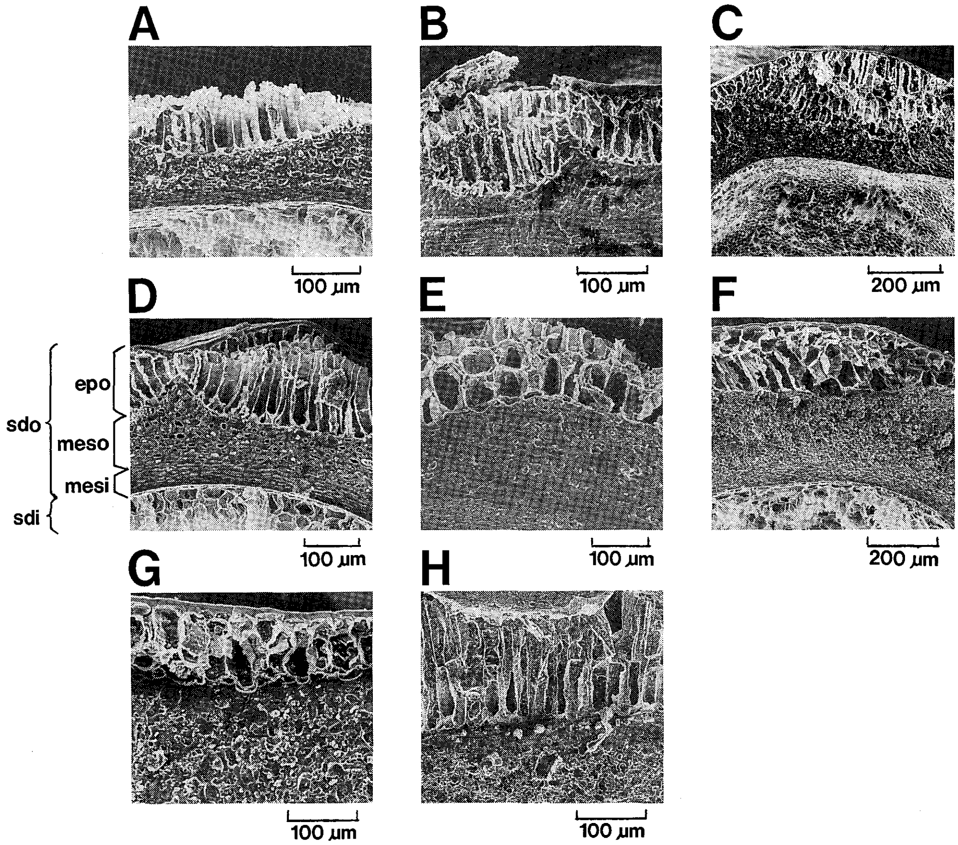

Fig. 4. Secondary Electron Images on the Transection of Seeds of Subgen. Fagara Species. A, *Z. schinifolium*; B, *Z. ailanthoides*; C, *Z. avicennae*; D, *Z. molle*; E, *Z. stenophyllum*; F, *Z. esquirolii*; G, *Z. nitidum*; H, *Z. dissitum*. (**epo**, outer epidermis; **mesi**, inner mesophyll; **meso**, outer mesophyll; **sdi**, inner seed coat; **sdo**, outer seed coat).

observed as 1.27 ± 0.03. The ratio of occupation of epidermis in outer seed coat was the lowest, Aepo/sdo and THKepo/sdo showing 20.6 ± 3.1 and 17.5 ± 2.7%, respectively. THKsdo/CRA was 35.4 ± 2.1%. Inner seed coat was made by 5–6 (1) cell layers and the ratio of thickness of inner seed coat to whole seed coat was slightly higher with the THKsdi/sd as 19.3 ± 6.1%.

19. Zanthoxylum dissitum Hemsl. (Figs. 2-H, 4-H)

Seeds were the biggest of all. Epidermis was made by 2–3 cell layers, R/T was 4.0–5.0, and the boundary between epidermis and sclerenchyma tissue was almost straight with the SFC value 1.27. THKsco/sci was 10.0–11.5 in sclerenchyma. Inner seed coat was made by 4–5 (1) cell layers and THKsdi/sd was relatively higher, 24.8%.

V. External morphology and botanical origin of commercial samples

1. "Chuan-jiaomu 川椒目"

External morphology: The fruit was yellowish brown. A small number of seeds existed of dehiscence pericarp or separated from the pericarp (Ratio of mixed pericarp was 75%). The shape of the seed was nearly glo-

Table 4-1. Anatomical characteristics of seeds of subgenera Zanthoxylum and Fagara species (1)

	Subgenus	Zanthoxylum									
	Species	*Z. bungeanum*	*Z. simulans*	*Z. armatum* var. *armatum*	*Z. armatum* var. *subtrifoliatum*	*Z. acanthopodium* var. *timbor*	*Z. podocarpum*	*Z. dimorphophyllum* var. *dimorphophyllum*	*Z. dimorphophyllum* var. *spinifolium*	*Z. piasezkii*	*Z. piperitum*
	Japanese name				Fuyu-zansho						Sansho
	Locality	China, Japan	Japan	Nepal	China	China	China	China		China	Japan
	n	4	4	1	3	4	3	2		2	1
	Epidermis number of layers	1–2	2	1–2		1	1–2	1		1	1–2
	ratio of radial diameter to tangential diameter of cells*	3.0–5.0	2.0–3.2	2.0–4.0	3.5–5.4	3.0–4.0	3.6–5.0	1.6–2.5	1.0–1.6	2.8–6.7	4.0–6.5
	boundary between epidermis and sclerenchyma tissue outline	wavy	slightly wavy	slightly wavy		slightly wavy	slightly wavy	nearly straight		slightly wavy	wavy
	SFC value**	1.23 ± 0.05	1.22 ± 0.01	1.18	1.22 ± 0.01	1.22 ± 0.02	1.15 ± 0.01	1.15 ± 0.02		1.18 ± 0.04	1.28
Outer seed coat	Ratio of thickness of outer mesophyll to inner mesophyll	2.1–3.0	3.0–3.8	1.4–4.0	3.6–4.5	2.2–3.6	2.0–3.5	inner mesophyll absent		3.0–4.0	2.2–4.0
	Ratio of an area of epidermis to outer seed coat (%)**	43.3 ± 6.3	47.5 ± 3.7	44.5	38.7 ± 1.2	34.0 ± 5.0	36.1 ± 4.3	37.1 ± 2.5		46.4 ± 6.8	39.3
	Ratio of the mean thickness of epidermis to outer seed coat (%)**	39.7 ± 6.3	43.6 ± 3.5	39.8	34.4 ± 0.9	30.3 ± 4.8	32.2 ± 3.4	36.0 ± 2.4		43.5 ± 7.1	35.8
	Ratio of the mean thickness of outer seed coat to a radius of transection of seed (%)**	26.9 ± 2.4	27.5 ± 1.4	32.1	32.6 ± 2.1	30.4 ± 1.8	29.9 ± 4.6	9.3 ± 0.7		23.8 ± 0.8	26.2
Inner seed coat	Number of large sized cell layers	2–3	3–4	3–4		3–5	5–7	2–3	3–4	2–3	4–5
	Ratio of the mean thickness of inner seed coat to whole seed coat (%)**	11.4 ± 1.1	10.1 ± 3.3	11.3	16.9 ± 3.4	16.5 ± 2.3	12.7 ± 5.2	15.9 ± 3.0		13.1 ± 3.3	8.6

*As for the epidermis composed of 1–2 and 2–3 cell layers, the measurement is done in the part of 1 cell layer and outermost layer, respectively.
**These data are taken from image analysis; SFC = Perimeter^2/(4π × area); this value indicates waviness.

Table 4-2. Anatomical characteristics of seeds of subgenera Zanthoxylum and Fagara species (2)

	Subgenus	Zanthoxylum		Fagara							
	Species	*Z. piperitum* f. *inerme*		*Z. schinifolium*	*Z. ailanthoides*	*Z. avicennae*	*Z. molle*	*Z. stenophyllum*	*Z. esquirolii*	*Z. nitidum*	*Z. dissitum*
	Japanese name	Asakura-zansho	Budo-zansho	Inu-zansho	Karasu-zansho						
	Locality	Japan	Japan	China	China	China	China	China	China	China	China
	n	1	4	2	2	3	3	1	1	3	1
Outer seed coat	Epidermis number of layers	1–2	1–2	1–2	1–2	2	1	2	2	1	2–3
	ratio of radial diameter to tangential diameter of cells*	2.2–4.0	2.0–4.0	3.5–4.5	5.0–11.0	2.5–3.5	5.5–9.3	1.4–1.6	2.5–3.0	1.8–3.0	4.0–5.0
	boundary between epidermis and sclerenchyma tissue outline	wavy		wavy	wavy	wavy	wavy	wavy	wavy	wavy	nearly straight
	SFC value**	1.26	1.26 ± 0.01	1.36 ± 0.03	1.27 ± 0.01	1.38 ± 0.09	1.24 ± 0.03	1.30	1.23	1.27 ± 0.03	1.27
	Ratio of thickness of outer mesophyll to inner mesophyll	4.3–5.5	ca 2.0	2.5–3.5	1.3–3.0	2.0–4.0	2.1–2.6	2.6–4.5	3.0–4.0	4.0–5.0	10.5–11.5
	Ratio of an area of epidermis to outer seed coat (%)**	37.8	49.1 ± 5.4	38.2 ± 0.2	52.2 ± 0.1	55.0 ± 3.2	52.8 ± 0.8	44.5	51.3	20.6 ± 3.1	41.8
	Ratio of the mean thickness of epidermis to outer seed coat (%)**	33.8	45.0 ± 5.6	35.9 ± 0.3	47.9 ± 0.1	50.2 ± 2.8	48.8 ± 0.5	39.8	48.2	17.5 ± 2.7	36.5
	Ratio of the mean thickness of outer seed coat to a radius of transection of seed (%)**	27.8	28.7 ± 1.7	18.7 ± 3.5	29.4 ± 0.2	27.3 ± 0.7	26.5 ± 1.4	32.4	21.8	35.4 ± 2.1	37.3
Inner seed coat	Number of large sized cell layers	3–4		5	2–3	5–6	6–7	5–6	3–5	5–6	4–5
	Ratio of the mean thickness of inner seed coat to whole seed coat (%)**	12.5	12.6 ± 0.9	13.4 ± 0.7	8.3 ± 0.3	10.6 ± 1.7	29.8 ± 5.3	14.8	19.9	19.3 ± 6.1	24.8

*As for the epidermis composed of 1–2 and 2–3 cell layers, the measurement is done in the part of 1 cell layer and outermost layer, respectively.
**These data are taken from image analysis; SFC = Perimeter^2/4π × area); this value indicates waviness.

Table 5. Key for the identification of *Zanthoxylum* species based on the anatomical characteristics of seeds*

1. Outer seed coat is thick and THKsdo/CRA** is above 18.7 ± 3.5%. Sclerenchyma of outer seed coat is divided into outer mesophyll [layer of cells of about same diameter] and inner mesophyll [layer of elongated cells]
Epidermis is 1, 2 or 3 cell layers
    2. Epidermis is thin and single cell layer. THKepo/sdo** is 17.5 ± 2.7% .... *Z. nitidum*
    2. Epidermis is thick and 1, 2 or 3 layer. THKepo/sdo is above 30.3 ± 4.8%
        3. Inner seed coat is thick and THKsdi/sd** is above 24.8%
            4. Seed is large and the epidermis is 2–3 cell layers. Outer mesophyll is thicker than inner mesophyll in sclerenchyma tissue. THKsco/sci** is 10.5–11.5. The large cell of inner seed coat are 4–5 cell layers .... *Z. dissitum*
            4. Seed coat is small and the epidermis is 1 cell layer. THKsco/sci is 2.1–2.6. The large cells of inner seed coat are 6–7 cell layers .... *Z. molle*
        3. Inner seed coat is thin and THKsdi/sd is below 19.9%
            5. THKsdo/CRA is 18.7 ± 3.5% .... *Z. schinifolium*
            5. THKsdo/CRA is above 21.8%
                6. The epidermal cells are radially short and R/T is 1.4–1.6 .... *Z. stenophyllum*
                6. The epidermal cells are radially long and R/T is above 2.0
                    7. The epidermis is 2 cell layers
                        8. Outer layer cells are about square and inner layer cells are rectangle radially elongated in epidermal cell .... *Z. esquirolii*
                        8. Together 2 cells are same shape and long radial in epidermal cell
                            9. The large cell of inner seed coat are 5–6 cell layers. Ratio of epidermis is large in outer seed coat and Aepo/sdo** is 55.0 ± 3.2% .... *Z. avicennae*
                            9. The large cells of inner seed coat are 3–4 cell layers. Aepo/sdo is 47.5 ± 3.7% .... *Z. simulans*
                    7. Epidermis is 1–2 cell layers
                        10. THKsdi/sd is 8.3 ± 0.3% .... *Z. ailanthoides*
                        10. THKsdi/sd is above 8.6%
                            11. The large cells of inner seed coat are 5–7 cell layers .... *Z. podocarpum*
                            11. The large cells of inner seed coat are below 5 cell layers
                                12. The large cells of inner seed coat are 2–3 cell layers
                                    13. THKsdo/CRA is 23.8 ± 0.8% .... *Z. piasezkii*
                                    13. THKsdo/CRA is 26.9 ± 2.4% .... *Z. bungeanum*
                                12. The large cells of inner seed coat are above 3 cell layers and no 2 cell layers
                                    14. Aepo/sdo is 34.0 ± 5% .... *Z. acanthopodium* var. *timbor*
                                    14. Aepo/sdo is above 37.9%
                                        15. THKsdo/CRA is 32.6 ± 2.1%. Aepo/sdo is 37.5–44.5%
                                            16. THKsdi/sd is 11.3% .... *Z. armatum* var. *armatum*
                                            16. THKsdi/sd is 16.9 ± 3.4% .... *Z. armatum* var. *subtrifoliatum*
                                        15. THKsdo/CRA is 26.2–30.4%. Aepo/sdo is 39.3–54.5%
                                            17. R/T is above 4.0 THKsco/sci is 2.2–4.0 .... *Z. piperitum* f. *piperitum* (Sansho)
                                            17. R/T is below 4.0
                                                19. THKsco/sci is 4.3–5.5 .... *Z. piperitum* f. *inerme* (Asakura-zansho)
                                                19. THKsco/sci is nearly 2.0 .... *Z. piperitum* f. *inerme* (Budo-zansho)

1. Outer seed coat is thin and THKsdo/CRA is 9.3 ± 0.7%. In outer seed coat, sclerenchyma cells are of same diameter and the epidermis is single cell layer
    20. Epidermal cells are long and R/T is 1.6–2.5 .... *Z. dimorphophyllum* var. *dimorphophyllum*
    20. Epidermal cells are short and R/T is 1.0–1.6 .... *Z. dimorphophyllum* var. *spinifolium*

*Central transection of seed was observed.

**A: area, CRA: average radius, R: radial diameter, T: tangential diameter, THK: average thickness, epo: outer epidermis, sci: inner mesophyll of sclerenchyma in outer seed coat, sco: outer mesophyll of sclerenchyma in outer seed coat, sd: seed coat, sdi: inner seed coat, sdo: outer seed coat.

bose. The surface of the seed was black, luster and slightly wrinkled. The length and width were 3.3–4.0 and 2.4–2.7 mm, respectively.

Internal structure: The seed completely corresponded with *Z. schinifolium.*

2. "Jiaomu 椒目"

External morphology: The seed was ellipsoidal and a part of seeds were half globose. The sample No. 15337 all had epidermis, while about 50% of the sample Nos. 13926 and 15459 peeled off epidermis. The surface of the seed was black and luster but not wrinkled for those sample where epidermis is observed, while those sample without epidermis showed dark brown, tarnish and not smooth. The length and width of the seed were 3.7–4.8 and 2.5–3.1 mm, respectively. A few sample were found to be mingled with the sample having reddish brown pericarp.

Internal structure: The seeds of the sample Nos. 13926 and 15337 completely corresponded with the *Z. bungeanum* and the sample No. 15459 was found to be a mixture and agreed with Budo-zansho and Asakura-zansho.

3. "Buguzhi 補骨脂"

External morphology: The seeds were ellipsoidal and a part of seeds was half globose. Epidermis was almost absent. The surface of the seed was black but not lustrous. The length and width of seed were 3.9–4.7 and 2.7–3.1 mm, respectively.

Internal structure: The seed completely corresponded with *Z. bungeanum.*

Results and Discussion

1. We observed the following characteristics: number of cell layers of epidermis of the outer seed coat, ratio of radial diameter to tangential diameter of epidermal cells, the value of SFC, ratio of average thickness of outer mesophyll to inner mesophyll in sclerenchyma, ratio of area of epidermis and thickness of epidermis in outer seed coat, ratio of thickness of outer seed coat to average radius of seed, number of cell layers of inner seed coat, ratio of thickness of inner seed coat to seed coat (Table 4). By the characteristics mentioned above, we could distinguish them from each other. The characteristics of 16 species three varieties and one form of the genus *Zanthoxylum* are summarized in Table 5.

2. Based on the above results we could clarify that "Chuan-jiamou" sample from Huhehaote, China was the fruits of *Z. schinifolium*, "Jiaomu" samples from Sanxi and Shanghai, and "Buguzhi" from Qinghai, China were the seeds of *Z. bungeanum*, "Jiaomu" sample from Osaka, Japan, was the seeds of "Budo-zansho" and "Asakura-zansho" (*Z. piperitum* f. *inerme*). "Buguzhi", properly, is the fruits of *Psoralea corylifolia* L., Leguminosae. "Jiaomu" and "Buguzhi" are similar in their external morphology but they belong to different families. So it seems a mistake to consider "Buguzhi" as "Jiaomu".

3. In the previous paper we showed that the main botanical origin of "Huajiao" were *Z. bungeanum* and *Z. armatum* var. *subtrifoliatum* (Ito et al 1996). Besides this we also found the fruits of *Z. schinifolium* as a commercial sample in Northern side, used for the "Huajiao" (Ito et al 1996). It has been reported that the botanical origin of "Jiaomu" is same as "Huajiao". In addition, *Z. armatum* growing in China (= *Z. armatum*) var. *subtrifoliatum* has been reported as the botanical origin of "Jiaomu". However, in the present study, we did not observe *Z. armatum* var. *subtrifoliatum* together with other *Zanthoxylum* spp. as the botanical origin except *Z. schinifolium* and *Z. bungeanum.*

4. The results of this experiment clearly suggested that those samples which were difficult to prepare slices by cutting with a knife could be studied by scanning electron microscope to determine the anatomical structure, which was very useful tool in the pharmacog-

nostical study.

5. This method was found to be superior than usual anatomical methods, which the data on size and shape of cells could be calculated by image analysis system.

We thank Prof. C. J. Huang and Prof. H. S. Chen, South China Institute of Botany, Academia Sinica for identifying some specimens from China. We are also grateful to Professors and staff of China Pharmaceutical University for their help during material collection.

References

All China Association of Traditional Chinese Medicine, Wuhan branch, edited by Association of Materia Medica, 1984. Handbook for diagnoses of Chinese Medicinal Herbs of Hubei (河北中草薬鑑別手冊), p. 188 (in Chinese).

Anhuisheng Health Office of Revolution Committee Editorial Office. 1975. Chinese Medicinal Herbs of Anhui (安徽中草薬), Anhui People's Press, Gefei, p. 353 (in Chinese).

Corner E. J. H. 1976. The Seeds of Dicotyledons. Cambridge University Press, Cambridge, I, p. 237, II, p. 422.

Economic Plants of Shandong Editorial Office. 1978. Economic Plants of Shandong (山東経済植物), Shandong People's Press, Jinan, p. 240 (in Chinese).

Fujian Institute of Traditional Medical and Pharmaceutical Sciences. 1984. Manual of Materia and Medica in Fujian (福建薬物誌), Vol. 2, Fujian Sci. and Tech. Pub. House, Fuzhou, p. 516 (in Chinese).

Guangxi Institute of Traditional Medical and Pharmaceutical Sciences. 1986. Manual of Medicinal Plants of Guangxi (広西薬用植物名録), Guangxi People's Press, Nannin, p. 313 (in Chinese).

Hebeisheng Health Office of Revolution Committee et al. 1977. Chinese Medicinal Herbs of Hebei (河北中草薬), Hebei People's Press, p. 561 (in Chinese).

Handbook of Medicinal Herbs of Ningxia Editorial Office. 1971. Handbook of Chinese Medicinal Herbs of Ningxia (寧夏中草薬手冊), Ningxia People's Press, Yinchuan, p. 373 (in Chinese).

Handbook of Medicinal Herbs of Shandong Editorial Office, 1970. Handbook of Chinese Medicinal Herbs of Shandong (山東中草薬手冊), Shandong People's Press, Jinan, p. 769 (in Chinese).

Health Bureau of Xizang Autonomous Region et al. 1971. The Useful Materia Medica of Tibe t (西蔵常用中草薬), Tibet People's Health Pub. House, Lhasa, pp. 193, 581 (in Chinese).

Hu S. L. 1989. The Authentic and Superior Medicinal Herbals in China (中国道地薬材), Heilongjiang Sci. and Tech. Pub. House, Haerbin, p. 182 (in Chinese).

Institute of Chinese Medicine, Branch Office in Shanxi, Chinese Academy of Medical Science. 1961. Manual of Shanxi Medicines (陝西中薬誌), Shanxi People's Press, Xia, p. 487 (in Chinese).

Institute of Materia Medica, Chinese Academy of Medical Sciences et al. 1961. Manual of Chinese Medicines (中薬誌), Vol. 2, The People's Health Pub. House, Beijing, p. 199 (in Chinese).

Ito C., Komatsu K., Liu Y. P., Shi D. W. and Namba T. 1996. Pharmacognostical studies on the Sino-Japanese crude drugs, "Huajiao" and "Sansho" (Part 2). On the botanical origins of Chinese crude drug, "Huajiao", Tibetan crude drug, "gYer-ma" and Ayurvedic crude drug, "Tumburu" derived from subgenus *Zanthoxylum*. Nat. Med. **50**: 328–343.

———, ———, ———, ——— and ———1997. Pharmacognostical studies on the Sino-Japanese crude drugs, "Huajiao" and "Sansho" (Part 3). On the botanical origin of Chinese "Huajiao" derived from subgenus *Fagara*. Nat. Med. **51** (in press).

Jiangsu Institute of Botany et al. 1991. Xinghua Bencao Gangyao (新華本草綱要), Vol. 2, Shanghai Sci. and Tech. Pub. House, Shanghai, pp. 260, 264 (in Chinese).

Jiangsu New Medical College. 1977. Chinese Materia Medica (中薬大辞典), Shanghai People's Pub. House, Shanghai, p. 2292 (in Chinese).

Komatsu K., Ito C., Liu Y. P. and Namba T. Nat. Med. **49**: 137–147 (1995).

Manual of Medicinal Plants of Zhejiang Editorial Office. 1980. Manual of Medicina Plants of Zhejiang (浙江薬用植物誌). Vol. 1, Zhejiang Sci. Tech. Pub. House, Hangzhou, p. 675 (in Chinese).

Mori T. (rev. ed.) 1972. Ben-cao-jing Ji-zhu (集注本草経), Vol. 5, South Osaka Printing Center, Osaka (in Chinese).

Quanguoyao Zhongcaoyao Huibian Editorial Office. 1978. The Compilation of Chinese Medicinal Herbs (全国中草薬彙編), The People's Health Pub. House, Beijing, Vol. 1, p. 447; Vol. 2, pp. 224, 258, 343, 564 (in Chinese).

Shanxisheng Health Office of Revolution Committee. 1972. Chinese Medicinal Herbs of Shanxi (山西中草薬), Shanxi People's Press, p. 343 (in Chinese).

Sichuansheng Chinese crude drug store of Chengdu. 1971. The Compilation of Medicinal Herbs Produced in Chengdu Area of Sichuan Prov. (四川省成都地区中薬材生産資料彙編), p. 449 (in Chinese).

劉 玉萍[a], 伊藤 親[b], 小松かつ子[b], 谿 忠人[c], 施 大文[d], 難波恒雄[b]: **「花椒」及び「山椒」の生薬学的研究（第4報）走査顕微鏡による「椒目」の基源解明**

「椒目」は利水薬として用いられている漢薬で，原植物は「花椒」と同じくミカン科のサンショウ(*Zanthoxylum*)属の種子に由来するとされている．*Zanthoxylum*属植物の種子は外形が類似している

ため同定が難しく，また内部形態においても種皮が堅いため切片の作製が困難である．そこで本報では，「椒目」の原植物を明らかにする方法の開発を目的に中国，日本及びネパール産の16種3変種1品種の種子について走査型電子顕微鏡及び画像解析装置を用いて組織学的研究を行った．その結果，各種は外種皮においては表皮細胞の層数及び放射方向径と接線方向径の比率，表皮と厚壁組織の境界が描く閉曲線の形とその周辺凹凸度，厚壁組織の外層と内層の平均の厚さの比率，外種皮中で表皮の占める割合，種子の半径に対する外種皮の厚さの割合などに種間差が認められ，それぞれ同定可能となった．また，上海と山西省大同市場品は*Z. bungeanum*の種子，内蒙古フフホト市場品は*Z. schinifolium*の種子と果皮の混合品であることが明らかになった．

([a]中医研究院中薬研究所，
[b]富山医科薬科大学和漢薬研究所，
[c]住友金属工業ハイクォリティライフ研究所，
[d]上海医科大学)